RBE of Energetic Iron Ions for the Induction of Early and Late Chromosome Aberrations in Different Cell Types

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BACKGROUND

Numerous published studies have reported the Relative Biological Effectiveness (RBE) values for chromosome aberrations induced by charged particles of different LET. The RBE for chromosome aberrations in human lymphocytes exposed *ex vivo* has been suggested to show a similar relationship as the quality factor for cancer induction. Therefore, increased chromosome aberrations in the astronauts' white blood cells post long-duration missions are used to determine the biological doses from exposures to space radiation. However, the RBE value is known to be very different for different types of cancer. Previously, we reported that, even though the RBE for initial chromosome damages was high in human lymphocytes exposed to Fe ions, the RBE was significantly reduced after multiple cell divisions post irradiation. To test the hypothesis that RBE values for chromosome aberrations are cell type dependent, and different between early and late damages, we exposed human lymphocytes *ex vivo*, and human mammary epithelial cells *in vitro* to various charged particles. Chromosome aberrations were quantified using the samples collected at first mitosis post irradiation for initial damages, and the samples collected after multiple generations for the remaining or late arising aberrations. Results of the study suggested that the effectiveness of high-LET charged particles for late chromosome aberrations may be cell type dependent, even though the RBE values are similar for early damages.

MATERIALS AND METHODS

Peripheral whole blood was collected from two healthy donors in vacutainer cell tubes containing sodium citrate. Peripheral blood mononuclear cells (PBMCs) were immediately separated by centrifugation, washed twice with PBS, counted and resuspended in RPMI1640/2mM Glutamine/10%FBS. Human mammary epithelial cells (CH184B5F5/M10) were cultured to confluence in DMEM medium with supplement of 10%FBS. Cells were exposed *in vitro* to Fe ions or protons (600MeV/nucleon) at NASA Space Radiation Laboratory (NSRL) at Brookhaven National Laboratory. In another experiment, primary PBMCs were exposed to 600 MeV/u Fe ions and 290 MeV/u Carbon ions at HIMAC, Chiba, Japan. PBMCs were also exposed to γ -rays acutely using a Cs source at NASA.

After exposure, PBMCs were stimulated to grow in medium containing 1% Phytohemagglutinin (PHA) and the epithelial cells were subcultured continuously to enable growth. Chromosomes were collected within the first cell cycle post irradiation and after multiple cell doublings using Calyculin-A.

Chromosomal aberrations were analyzed using fluorescent *in situ* hybridization (FISH) with whole chromosome probes for chromosomes 3 and 6 (MetaSystems). In addition, chromosome 3 was painted with the *XCyte3* mBAND kit (MetaSystems) which distincts the chromosome in 23 different color bands.

mBAND and FISH

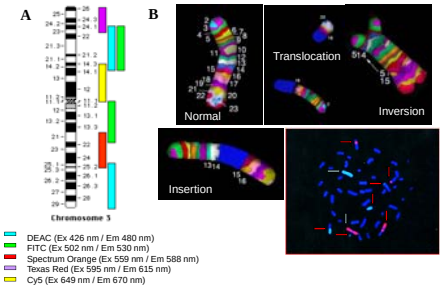

Figure 1. Examples of chromosome 3 of human lymphocytes painted with mBAND after 4 Gy γ-ray exposure. Figure 2. Example of two pairs of chromosomes in human lymphocyte painted with two color whole chromosome probes. Aberrant chromosome pieces pointed using red arrow (grey arrows indicating normal chromosomes) include translocations, insertion, and deletion.

RESULTS

1. Cell growth of irradiated lymphocytes and epithelial cells

Cell samples collected at 8-13 days post Fe ion and proton irradiation were used to analyze chromosome aberrations in lymphocytes and epithelial cells using FISH technique (mostly at 5-7 doublings post irradiation). Lymphocytes were also analyzed at 7 and 14 days post Fe ion and γ–irradiation using mBAND technique at the similar doublings of the cell population.

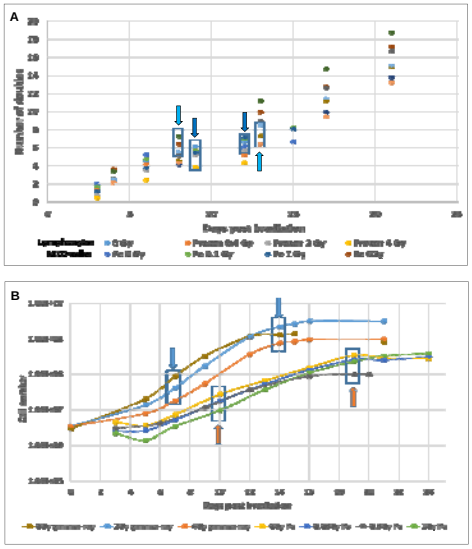

Figure 3. (A) Cell growth of lymphocytes and M10 cells after Fe ion or proton irradiation; (B) Cell growth of lymphocytes after Fe ion or gamma ray irradiation. The chromosome aberrations were analyzed using cell samples with the arrow marker.

2. Fractions of cells containing initial or late chromosomal aberrations induced by Fe ions or Protons

At multiple cell doublings post Fe ion-irradiation, only a small fraction of cells contained aberrations (~10% of the initial fraction). In comparison, a significantly greater fraction of epithelial cells contained late aberrations (~50% of the initial fraction). For low-LET protons, 40-50% of the cells containing initial damages remained after multiple cell divisions for both cell types.

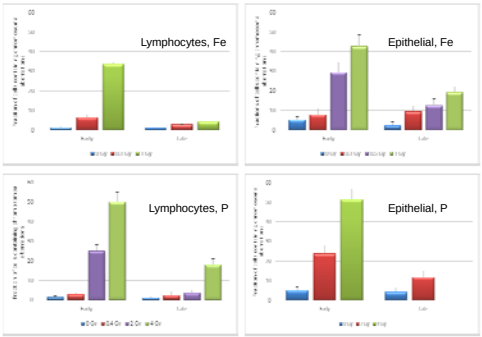

Figure 4. Initial (Early) and late chromosomal damages in lymphocytes (left) and epithelial cells (right) after Fe ion (upper) and proton (lower) irradiation. The data are presented as fraction of cells containing chromosomal aberrations.

3. Fractions of lymphocytes containing different number of radiation-induced chromosome breaks

The number of chromosome breaks on chromosome 3 and 6 were evaluated to quantify the severity of radiation-induced damages. Most severely damaged cells (containing 4 or more breaks) were eliminated in later generations, resulting in a low yield of late aberrations for Fe ions. Compared to protons, Fe ions induced more severe chromosomal aberrations initially. More than15% of the damaged cells contained 4+ breaks after Fe ion irradiation.

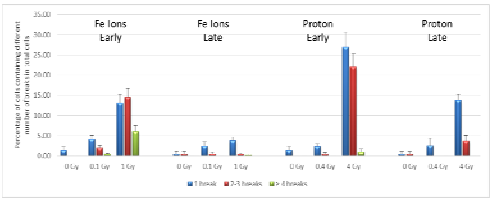

Figure 5. Fraction of lymphocytes containing different number of chromosome breaks after Fe ion (left) and proton (right) irradiation.

4. Initial and late chromosome aberrations in irradiated lymphocytes using mBAND technique

The data analyzed using mBAND technique of lymphocytes post γ– and Fe ion irradiation were consistent with FISH analysis.

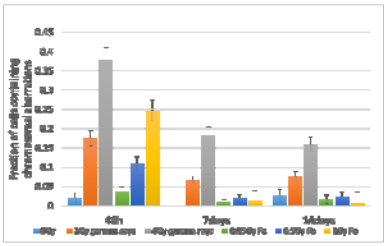

Figure 6. Fraction of cells containing initial and late chromosomal damages in total cells.

5. Effectiveness of Fe ions to protons for early and late chromosome aberrations

Based on the data analyzed in this study, it suggests that the late RBE of Fe ion on M10 cells may be same as that based on initial damage (about 4). However, for lymphocytes, the late RBE may be altered and more correspondent to late effect of radiation.

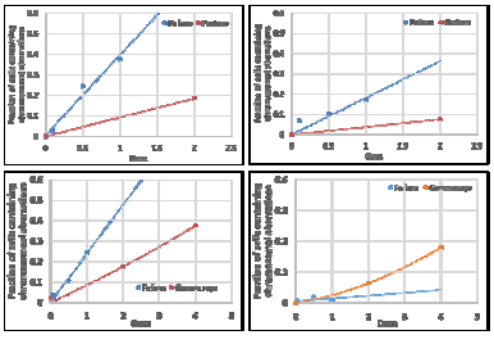

Figure 8. The percentage of cells containing initial or late chromosome damage in epithelial cells, showing a similar effectiveness between early and late damages for Fe ions in comparison to protons..

CONCLUSIONS

- For low-LET radiation, a significant fraction of the initially damaged cells survived after multiple cell divisions, regardless of the cell types. However, the fraction of survival cells with initial chromosome damages induced by high-LET Fe ions appeared to be cell type dependent. The fraction was low for primary human lymphocytes, but significantly higher for the CH184B5F5/M10 human epithelial cells.
- The cells that didn't survive after multiple generations usually contained chromosome aberrations involving a large number of chromosome breaks.
- Due to the difference in the fraction of surviving cells after high-LET radiation exposure, the RBE for late chromosome aberrations can be different for different cell types, even though the RBE values are similar for early damages. Such a difference may explain the different RBE values for late effects such as cancer between different organs.

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